Animal models of osteoarthritis: lessons learned while seeking the 'Holy Grail'

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Purpose of review

Difficulties in studying osteoarthritis in humans that stem from both the low sensitivity of diagnostic tools and the low availability of diseased tissues explain why research on animal models remains highly dynamic. This review will summarize the recent advances in this field.

Recent findings

With regard to the etiology of osteoarthritis, synovial macrophages mediate osteophyte formation, whereas increased ligament laxity could be responsible for spontaneous osteoarthritis in guinea pigs. The concomitant changes in subchondral bone and cartilage reported in several models, and the structure-modifying effects of some bone inhibitors have confirmed the importance of bone in osteoarthritis. With regard to cartilage pathobiology, ADAMTS-5 is the major aggrecanase responsible for cartilage destruction, whereas inadequate control of oxidative stress and decreased expression of transforming growth factor-β receptors could predispose to osteoarthritis. New models include a postmenopausal rat model, the groove model and a joint-specific bone morphogenetic receptor-deficient mouse. The iodoacetate model was also validated as the first pain model of osteoarthritis.

Summary

In view of the multiple animal models available, there is a need to reach a consensus on one or several gold standard animal model(s). New studies indicate that important differences in therapeutic response exist between young and old animals, and between spontaneous and surgical models, suggesting that not all models are adequate models of osteoarthritis.

Keywords

animal models, bone, 'gold standard', osteoarthritis

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Abbreviations

ACLT	anterior cruciate ligament transection
BMP	bone morphogenetic protein
FGF	fibroblast growth factor
GH	growth hormone
IL	interleukin
MMP	matrix metalloproteinase
NO	nitric oxide
OA	osteoarthritis
OVX	ovariectomy
ROS	reactive oxygen species
TGF	transforming growth factor

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Introduction

Animal models of osteoarthritis (OA) include spontaneous models in aging animals, genetically modified mice, as well as surgically, enzymatically or chemically induced models (see [1,2] for recent reviews). Research on these models remains highly dynamic due to the difficulties in studying osteoarthritis in humans, which stem from poorly sensitive diagnostic tools and the low availability of diseased tissues. This review summarizes the recent advances in this field and underlines the impact of the major new findings on OA research.

Old and new models: back to the future!

Established animal models of OA continue to be widely used as tools to evaluate and improve various diagnostic techniques used to study OA. These range from magnetic resonance imaging and other imaging modalities [3–8] to tissue engineering methods [9–12] and establishment of biochemical markers [13–15]. To facilitate the comparison of the different existing models, a novel OA cartilage scoring system, potentially easier and more reliable to use, was developed [16•]. Among the surgically induced animal models of OA, the anterior cruciate ligament transection model (ACLT) in dogs and the partial menisci resection model in rabbits have been historically widely used [1]. These models continue to be investigated in these species [17] as well as others such as the sheep [18], the cat [19•,20,21], or in smaller and cheaper animals such as the guinea pig, the rat and very recently, for the first time, in the mouse [22•,23]. In the mouse, using various combinations of ligament transection and menisectomy, four models exhibiting various OA severity (mild, moderate and severe) and direction of instability (anterior versus medial) were created, and the progression of cartilage destruction

and osteophyte formation described up to 8 weeks post-surgery [22•].

The use of the ovarectomized rat as an animal model of OA was recently reported. Ovariectomy (OVX)-induced estrogen deficiency, in 7-month-old Sprague-Dawley rats, resulted in mild erosions of knee articular cartilage 9 weeks after surgery without any further noticeable progression 6 weeks later [24]. This cartilage erosion was paralleled by a transient increase in urinary CTX-II, a biomarker of type II collagen catabolism, in the first 4–6 weeks after surgery. As the rats were not pair-fed, it remains to be seen whether the transient degradation of articular cartilage was due to the weight gain (an important risk factor for OA) induced by the estrogen deficiency or to the estrogen deficiency itself. In any case, treatment with estrogen or a selective estrogen receptor modulator prevented the OVX-induced cartilage damage as well as the CTX-II increase. Similar OVX-induced joint changes were previously described in ewes and macaques (see [25,26•] and references therein). The transposition of the OVX model to the rat, however, constitutes an advance by providing a more rapid and affordable animal model of early stage OA, particularly relevant for post-menopausal OA. It is also an interesting model to better understand the role of bone in OA pathology as this model is the gold standard animal model for postmenopausal osteoporosis and, as a consequence, the OVX-induced bone changes are already well described. Overall, the OVX-rat is a new, cheap, rapid and convenient animal model of early postmenopausal OA with a well-characterized osteoporotic phenotype. The OVX-induced changes could be mediated by a local increased release of nitric oxide (NO). Indeed, the observed phenotype in OVX-ewes is similar to the phenotype displayed by ewes after topical treatment with an exogenous NO donor [27,28] (the latter reference being a thorough investigation of the detrimental effects of an excessive amount of NO on bone and cartilage metabolism in the menisectomized ewe as well as an interesting discussion on the local and differential consequences of altered loading patterns on subchondral bone structure) and articular cartilage from OVX-ewes released more NO *ex vivo* while displaying increased cartilage immunostaining for inducible NO synthase and nitrotyrosine [26•].

Is osteoarthritis a bone disease, a cartilage disease, a synovial disease, a ligament disease or a little bit of everything?

The long-lived debate on whether OA is primarily a cartilage or a bone disorder has been invigorated by the recently documented acceleration of subchondral bone turnover in OA and by the interest of the pharmaceutical industry to evaluate whether antiresorptive bone drugs could be beneficial for OA treatment. Two recently developed models could become ideal research tools, after further characterization, to improve our understanding of the differences between articular cartilage-driven and bone-driven processes in OA. On the one hand, the canine groove model, in which the disease is induced by surgically made grooves in the articular cartilage [29•], constitutes an excellent model of primarily cartilage-driven disease. On the other hand, another canine model, in which a subchondral bone trauma is induced by transarticular impact, resulting in immediate subchondral bleeding, edema and trabecular microfractures, but not in any cartilage degeneration as assessed by magnetic resonance imaging [30–32] could constitute a model of primarily bone-driven disease once the absence of overt cartilage impairment a few hours to a few days after the impact has been confirmed by more sensitive methods. In addition to these two models, other animal studies comparing both cartilage and bone changes in parallel during OA progression are also useful to address the implication of these two tissues in the etiology of OA. Several new studies, using the spontaneous [33] and menisectomized guinea pig model [34], the ACLT and menisectomized rat models [35•], and the ACLT dog model [36,37] documented early and concomitant changes in articular cartilage and subchondral bone. In the two surgical rat models, cartilage damage and proteoglycan loss were observed as early as 1 week postsurgery, whereas subchondral bone loss could only be detected 2 weeks postsurgery [35•]. Although these last results could be interpreted as supporting an etiological role for cartilage in OA, they do not constitute an absolute demonstration as the techniques and endpoints used may have different sensitivities to molecular changes taking place in the two tissues. In the ACLT and menisectomized models (rat, guinea pig, rabbit and dog), the OA-induced bone remodeling was characterized by an early subchondral bone loss followed by a later increase in subchondral bone volume [6,34,35•,36,38]. The only exception was in the ACLT cat model, in which a long-term thinning of the subchondral plate was observed [19•]. This difference between models could be an imaging artifact due to the use of micro-computed tomography in the cat model – a method that does not distinguish compact mineralized bone from calcified cartilage. Confirmation of subchondral plate thinning in the ACLT cat by histomorphometry is thus required. If confirmed, it would question the importance of changes in the subchondral plate for OA progression and would suggest that changes in subchondral cancellous bone could be more relevant. Ex-vivo culture experiments on bone cells and explants obtained from ACLT dogs indicated that both subchondral plate and subchondral trabecular bone metabolism were altered by OA [39], confirming previous studies (see [19•] and references therein). Treatment with a NO donor compound increased subchondral sclerosis, suggesting a role for NO in this process [28]. In this context, alendronate, an inhibitor of bone

resorption, was shown to have chondro-protective effects including reduction of markers of cartilage degradation (CTX-II and cartilage oligomeric matrix protein) and a decrease in the formation of osteophytes in the rat ACLT model [40] (providing a proof-of-principle demonstration that inhibiting bone remodeling in OA can decrease osteophyte formation and have chondro-protective effects). While not formally addressing the question of the causative tissue in OA, this study, along with others [41,42], suggests that inhibitors of bone resorption could be used as structure-modifying agents even if doses higher than those used to treat osteoporosis might be required for that purpose. Interestingly, collagen turnover in the anterior cruciate ligaments of the Dunkin-Hartley guinea pig is increased before any of the previously reported OA-related changes in bone or cartilage [43••]. This increased collagen turnover is associated with an increased ligament laxity that could induce joint instability and cause the observed OA-associated bone remodeling. Indeed, altered loading is known to affect the thickness, turnover and mineral density of bone. This study suggests that anterior cruciate ligaments play a key role in the etiology of spontaneous OA and might be a highly relevant tissue target for the development of future therapies.

Decreased osteophyte formation and synovial fibrosis following in-vivo depletion of synovial macrophages in collagenase-induced OA elegantly demonstrated that synovial lining macrophages mediate osteophyte formation and synovial fibrosis [44]. The decreased formation of osteophytes following intra-articular injection of transforming growth factor (TGF)-β in mice depleted in synovial macrophages demonstrated more specifically that the osteophytes-inductive effects of TGF-β were mediated by macrophages [45] (nicely illuminating the mechanisms by which TGF-β induces osteophyte formation). Macrophage depletion decreased the TGF-β-induced production of bone morphogenetic protein (BMP)-2 and -4 in the synovium; however, the absence of effects of TGF-β on the production of BMPs by macrophages *in vitro* suggested that, *in vivo*, the TGF-β-induced production of BMPs is only indirectly mediated by macrophages. These two studies demonstrate that macrophages constitute a key cell target for therapeutic approaches aiming at preventing osteophyte formation. Taken together, it will be important to consider all tissues of the joint in seeking a gold standard animal model – the 'Holy Grail' of preclinical research in OA (see Discussion).

Pain

Despite the severe pain caused by OA resulting in poor quality of life for OA patients, characterization of animal models traditionally focused on structural damage and completely disregarded pain. As a consequence, no animal model of OA pain was available – a situation that seriously hampered research in this area. Recent studies indicated that iodoacetate injection into the rat knee joint very rapidly mimics the pain and biochemical/structural changes associated with OA [46–50], an important discovery that should boost research on OA pain in the future. Iodoacetate injection induced dose-dependent changes in hind paw weight distribution as well as marked mechanical hyperalgesia (i.e. extreme sensitivity to painful stimulus) and tactile allodenya (i.e. painful response to a normally innocuous stimulus). These changes were reversed or decreased with morphine, acetaminophen, nonsteroidal antiinflammatory drugs or cyclooxygenase inhibitors. While gait, mobility and structure changes induced by iodoacetate were previously reported (see [51] and references therein), the major contribution of these new studies is to demonstrate that in this rapid and affordable model, joint pain can be modulated as well as easily reproducibly quantified. The neuropeptide calcitonin gene-related peptide and the vanilloid receptor, TRPV1, could be involved in the sensation of pain in this model [52]. In STR/1N mice and collagenase-induced murine OA, production of prostaglandin E_2, a nociceptor activator, increased during the development of OA, thereby mimicking the human disease [53].

Of stress and death: role of oxidative stress and premature apoptosis

Observations have indicated that extracellular superoxide dismutase, a scavenger of reactive oxygen species (ROS), is decreased in human OA cartilage. This was also found in the cartilage of STR/ort mice before these mice displayed histological evidence of OA, suggesting that inadequate control of ROS could play a role in the initiation and pathophysiology of OA [54]. This increased oxidative stress could explain the increased level of apoptosis observed in the cartilage of these mice [55] and in aged rabbit cartilage [56]. In rats, the number of chondrocytes and the activity of the antioxidant enzyme catalase decreased with age, while the production of ROS and the vulnerability to ROS-induced toxicity increased, suggesting that oxidative stress could predispose the aging cartilage to OA [57•]. Similarly, ex-vivo spontaneous NO release from guinea pig knee cartilage increased steadily with age as OA developed [58]. In parallel, intracellular ATP levels in chondrocytes declined by 50%, whereas activity of the ATP-scavenging enzyme and extracellular levels of its end-product, inorganic pyrophosphate, increased, suggesting that NO might induce mitochondrial dysfunction and ultimately cartilage calcification in OA. In addition, topical treatment with a nitric donor compound induced cartilage changes in intact ewes [27] and increased subchondral bone sclerosis and cartilage degeneration in menisectomized ewes [28], suggesting that overproduction of

the ROS, NO, stimulates disease progression. This confirmed the previous protective results obtained in the ACLT dog with a selective inhibitor of inducible NO synthase [59]. The negative effect of oxidative stress in OA could be partly mediated by the formation of advanced glycation end-products, whose nonenzymatic formation includes an oxidation step. Interestingly in the ACLT dog, experimental induction of advanced glycation end-products production accelerated cartilage degradation [60] suggesting that accumulation of advanced glycation end-products is part of the molecular mechanisms by which aging predispose to the development of OA.

Supporting earlier animal studies, Galois *et al.* [61] reported that slight or moderate exercise, but not intense exercise, exerted a chondro-protective effect in the ACLT rat model. This effect could be related to a decreased level of chondrocyte apoptosis mediated by an exercise-induced overexpression of heat shock protein 70. In the iodoacetate and ACLT rat models, the expression of TRAIL, a proapoptotic cytokine of the tumor necrosis factor superfamily, and of the death receptor, DR4, was increased while the expression of the decoy death receptor, DcR1, was decreased. Taken together, this suggests that TRAIL-induced apoptosis may play a role in the pathogenesis of OA [62].

Enzymes: weapons of mass destruction for articular cartilage

Since the discovery a few years ago that several members of the ADAMTS (A Disintegrin And Metalloprotease with ThromboSpondin-like repeat) family of enzymes were able to cleave aggrecan at its known site of degradation in human joint diseases, the race was on to find which ADAMTS(s) was/were involved in OA cartilage destruction. Generation of mice deficient in ADAMTS-1 [63], -4 [64] and -5 [65••,66••] demonstrated that ADAMTS-5 was the major aggrecanase involved in this process. Indeed, compared to wild-type mice, ADAMTS-5-deficient, but not ADAMTS-1- or -4-deficient, mice were more resistant to aggrecan loss and articular cartilage destruction induced by joint instability or inflammatory challenge. Analysis *in vitro* revealed that deficiency in ADAMTS-5, but not in ADAMTS-1 or -4, completely prevented the interleukin (IL)-1-induced release of aggrecan from murine articular cartilage. These are the first reports to indicate that a single gene deletion can significantly slow down articular cartilage destruction in joint diseases. They identify ADAMTS-5 as a key target for the development of future chondro-protective drugs.

ADAM-15 is a membrane-anchored adamalysin metalloproteinase disintegrin upregulated in OA that contains an active metalloproteinase site. Until recently, it was thought to have a catabolic effect on cartilage metabolism; however, accelerated development of OA observed in male ADAM-15-deficient mice, as well as pro-adhesive and cell-survival-promoting effect *in vitro* on chondrocytes, indicated, on the contrary, a homeostatic role for ADAM-15 in cartilage metabolism [67]. The phenotypic similarity that ADAM-15-deficient mice share with mice deficient in α_1 integrin [68] suggested that the OA phenotype of the ADAM-15-deficient mice could be linked to the ability of ADAM-15 to regulate cell–matrix interactions. The absence of phenotype in females, its partial penetrance in males and its mild severity in comparison to the phenotype of the α_1 integrin-deficient mice, however, also indicated that the functional importance of ADAM-15 in cartilage homeostasis is moderate and modulated by mechanisms remaining to be investigated.

New animal studies further support a role for cathepsin K in OA and suggest that besides matrix metalloproteinases and aggrecanases, cathepsin K is also involved in cartilage degradation. Cathepsin K mRNA was transiently upregulated during early cartilage degeneration in the chondrocytes located close to the cartilage defects, both in the transgenic Del1 mouse (which harbors a short deletion mutation in a type II collagen transgene) and in their nontransgenic controls that developed OA at an older age [69]. Its mRNA and protein levels were also upregulated in the early osteoarthritic articular cartilage and subchondral bone of the ACLT dog [36,37]. Transgenic mice overexpressing cathepsin K in osteoclasts were known to develop high-turnover osteopenia in trabecular bone [70] and increased porosity in cortical bone [71]. These mice are now shown to also develop progressive synovitis, which, upon aging, resulted in the destruction of articular cartilage [72•]. As cathepsin K is expressed in synovium, cartilage and bone, three tissues involved in OA pathology, it would be of interest to generate tissue-specific transgenic mice in order to assess the relative importance that these tissues have in the detrimental role of cathepsin K in joint diseases. In addition, in view of the observed upregulation of cathepsin K in chondrocytes during early OA in different mouse models, the generation of mice with a deficiency in cathepsin K restricted to articular cartilage would confirm the direct catabolic role of cathepsin K in OA articular cartilage degradation. Nontissue-specific cathepsin K-deficient mice are available [73], but the development of osteopetrosis by these mice, a severe bone phenotype, will blur the physiological significance of any resistance towards OA that these mice can possibly display.

Growth factors, cytokines and extracellular matrix: molecular interplay?

Deficiencies in BMP receptor type 1a [74], Mig-6 [75••] or Bgn and Fmod [76,77•], two small leucine-rich proteoglycans, all resulted in an early, rapid and severe

development of OA, indicating that these molecules are essential for maintaining joint health and articular cartilage integrity. Altered synthesis and proteolysis of small leucine-rich proteoglycans in human OA and in the menisectomized sheep further confirmed the implication of small leucine-rich proteoglycans in OA (see [78] and references therein). Despite some differences in their phenotypes, these new genetically modified animal models could well be phenocopies, i.e. have their phenotypes linked molecularly [79]. Existence of a molecular link between the BMP receptor type 1a- and the Bgn- and Fmod-deficient mice is suggested by the defective binding and downstream signaling of BMP-2 and -4 in Bgn-deficient bone cells [80], and by the ability of BMP to partially rescue the gene expression changes induced by the Bgn deficiency [81]. A deeper understanding of the molecular overlap in the mechanisms leading to OA in these different models awaits further investigation. In addition to demonstrating the importance of BMP receptor type 1a in joint homeostasis, the paper by Rountree and collaborators [74] constitutes an important new step in the generation of genetically modified animal models of OA. These investigators were able to limit the BMP receptor type 1a deficiency to the joints, by using a Cre–lox system in which the expression of the Cre-recombinase was driven by the promoter of the growth differentiation factor-5, a gene whose expression is restricted to joint tissues. This spatially as well as temporally limited expression of the Cre-recombinase (growth differentiation factor-5 is expressed relatively late in development) allowed them to bypass the embryonic lethality observed in mice with a knockout. Such a strategy permits the study of the consequence in adults of deletions that are lethal for the embryos and also facilitates the physiological interpretation of the obtained phenotypes by limiting the pleiotropic effects commonly observed in knockout mice and full transgenics. As human OA results in tissue- and stage-specific disregulations, such animal models displaying time- and tissue-specific genetic anomalies mimic more closely the human disease and hence represent ideal genetically modified animal models for OA research.

IL-6 deficiency in male, but not female, mice resulted in bilateral cartilage destruction, medial subchondral sclerosis, decreased knee bone mineral density as well as increased proteoglycan deposition and ectopic bone formation in the medial collateral ligaments [82••] by 18 months of age. *Ex vivo*, decreased proteoglycan synthesis by IL-6-deficient articular cartilage was also observed. The fact that the cartilaginous part of the phenotype had the highest frequency and occurred bilaterally, contrary to the phenotypic changes observed in other joint tissues, suggested that in this model OA is a cartilage-driven process. The role of IL-6 in the pathophysiology of OA might be more important in old age because collagenase injections at a young age induced a similar degree of joint pathology in deficient and wild-type mice. One cannot, however, exclude that the intensity of the collagenase challenge might have masked differences in joint metabolism between the two genotypes. Altogether, these data suggest an overall protective role for IL-6 in male mice, although one should be cautious about such conclusions drawn from the phenotypic analyses of deficient mice. Indeed, gene deletion of either IL-1β, IL-1β converting enzyme, inducible NO synthase or matrix metalloproteinase (MMP)-3 all accelerated the development of knee OA in mice, spontaneously or after menisectomy and ligament transection – a counterintuitive result in view of the well-described ability of these molecules to induce degenerative change in cartilage [23]. This indicates that molecules commonly considered as detrimental in OA also have other important physiological functions and that their complete absence, instead of slowing down the disease progression as expected, on the contrary accelerates it.

Destruction of articular cartilage results from an imbalance between anabolism and catabolism in chondrocytes. With age, articular cartilage becomes less responsive to growth factors such as transforming growth factor (TGF)-β – a phenomenon that could induce OA by disturbing the anabolic–catabolic balance in articular cartilage. A recent study indicated that, in mice, the age-related reduced response to TGF-β was due to an IL- independent diminution in protein expression of TGF-β receptors I and II, and not to changes in protein levels of intracellular signaling or inhibiting Smad molecules [83•]. In parallel, the expression of TGF-β2 and 3 decreased with age. When an adenovirus expressing the TGF-β inhibitor, LAP, was injected into the joint, cartilage repair after IL-1 stimulation was decreased, further supporting the importance of TGF-β in articular cartilage homeostasis [83•]. This study suggests that strategies limited to the delivery of growth factors to the joint in order to induce cartilage repair might ultimately fail, especially if the responsiveness of the aging chondrocyte is not addressed in parallel. Apart from TGF-β, other important factors regulating the anabolic/catabolic balance in articular cartilage include growth hormone (GH), insulin growth factor-1 (produced primarily in response to GH stimulation) and fibroblast growth factors (FGFs). Transgenic mice expressing bovine GH developed accelerated OA [84]. Articular chondrocytes from these mice displayed a hyperactive metabolism, an increased tumor necrosis factor-α expression, a decreased proliferation rate and an increased level of apoptosis. This transgenic mouse could constitute a good model to study the role of GH and insulin growth factor-1 in cartilage metabolism and OA. Regarding FGFs, 1-day intra-articular administration of FGF-2 through an osmotic pump or autologous transplantation

of chondrocytes transfected with an adenovirus containing the FGF-2 gene caused regenerative repair of full-thickness defects of articular cartilage in rabbits [85,86]. As the relevance of these models of traumatic cartilage injury for degenerative OA is questionable, the recent confirmation of the potential therapeutic effects of growth factors in classical OA models constitutes an important piece of information. Sustained release of FGF-2 through the use of gelatin hydrogel microspheres stopped the OA progression in the ACLT rat model [87••], whereas FGF-18 stimulated cartilage repair in the menisectomized rat [88•]. As these studies used immature or fairly young adult animals, however, it remains to be seen whether these effects can be reproduced in old animals known to be less responsive to growth factors. Indeed, FGF-2 promoted the repair of partial thickness articular cartilage defects in immature rabbits, but not in mature rabbits [89]. Finally, in another recent study, local delivery of BMP-4 by genetically engineered muscle-derived stem cells from young mice resulted in enhanced chondrogenesis and significantly improved cartilage repair in rats [90••]. Although potential long-term ossification of the reparative tissue and use of muscle-derived stem cells from old donors remained to be evaluated [91], the use of muscle-derived stem cells constitutes an alternative to former similar approaches [92] that use periosteum-derived or bone marrow-derived stem cells.

Other molecular players

Upregulation of β-defensins, antimicrobial peptides of the innate immunity system, in early OA cartilage of the STR/ort mouse suggest that β-defensins also play a role in the pathophysiology of OA, possibly by linking inflammation and articular cartilage remodeling [93]. Protein expression of parathyroid hormone-related protein and of one of its major inducers, extracellular calcium-sensing receptor, by chondrocytes in early spontaneous guinea pig OA [94], as well as parathyroid hormone-related protein expression by proliferating chondrocyte clones in late OA in the menisectomized rabbit [95], suggests that parathyroid hormone-related protein is involved in OA progression.

In-vitro experiments demonstrated that activation of DDR-2 by intact type II collagen molecules increased expression of MMP-13 through the Ras/Raf/MEK/ERK pathway [96•]. Chondrodysplasic *Cho*/+ mice, which harbor a heterozygous mutation resulting in a premature termination of the translation of the α1 chain of type XI collagen, have thicker collagen fibrils around the chondrocytes and upregulated levels of DDR-2 and MMP-13 in cartilage before displaying an increased type II collagen catabolism in this tissue [96•,97]. Taken together, these data suggest that cartilage destruction in these mice result from the DDR2-mediated MMP-13 induction caused by the enhanced exposure of chondrocytes to type II collagen as a result of the decreased amount of type XI collagen in the mutant cartilage [96•].

Drugs that ameliorate osteoarthritis

Licofelone, a lipoxygenase and cyclooxygenase inhibitor, which slowed down early cartilage destruction in the ACLT dog model [98], decreased the protein levels of MMP-13, cathepsin K, ADAMTS-4, ADAMTS-5 and 5-LOX in articular cartilage [37]. It also prevented early loss of calcified cartilage and subchondral bone, while decreasing the protein levels of MMP-13 and cathepsin K in bone cells [36]. In the same model, PD-0200347, an $\alpha_2\delta$ ligand of voltage-activated Ca^{2+} channels, decreased cartilage destruction, as well as the protein expression of MMP-13 and inducible NO synthase protein in articular cartilage [99•]. In the menisectomized guinea pig model, the use of a wide-spectrum MMP inhibitor, S-34219, decreased cartilage destruction, but not glycosaminoglycan loss, emphasizing the importance of dual inhibition of aggrecanases and collagenases for the prevention of cartilage destruction in OA [100•]. In the same model, treatment with pioglitazone, a synthetic peroxisome proliferator-activated receptor-γ agonist, decreased articular cartilage destruction and the cartilage levels of MMP-13 and IL1-β [101•]. As pioglitazone is already commercialized as an antidiabetic drug (although at a lower dosage than used in this study), it might be particularly useful for the treatment of OA patients with type II diabetes. In the ACLT rabbit, intra-articular injections of dehydroepiandrosterone, an adrenal androgen, decreased cartilage destruction [102]. It decreased MMP-1, MMP-3 and IL-1β mRNA production in cartilage, while increasing tissue inhibitor of MMP-1 mRNA levels. Glucosamine–HCl in the ACLT rabbit had a debatable partial protective effect on the lateral cartilage [103,104]. In a rat study comparing different molecular weight hyaluronan solutions, only the most elastoviscous high-molecular-weight hyaluronan (Synvisc) reduced the perception of nociceptive stimuli, suggesting that elastoviscosity is a key parameter in determining the analgesic properties of viscosupplementation materials [105]. In the ACLT dog, intra-nasal administration of calcitonin decreased cartilage degradation and net loss of collagen with no effect on the size of osteophytes [106].

Putting it all together: the road lying ahead...

Enhanced research activity in the field has resulted in the development of numerous animal models of OA; however, no consensus currently exists regarding which model and species is the most relevant for human OA. Lack of such a gold standard animal model of OA originates from a poor understanding of the disease etiology, from a lack of knowledge on the differences

between the different components of the OA syndrome and from the absence of clearly effective structure-modifying drugs in humans that could be used to evaluate the relevance of the existing animal models. Hence, while the diversity of the available models provides us with a wealth of valuable information on joint biology and OA pathology, differences between models also makes comparisons between studies sometimes difficult and the transposition of animal results to human OA hazardous. In this context, the validation of a gold standard animal model of OA and its consensual recognition by the research community would constitute a major advance in the field. This quest of the 'Holy Grail' is the most relevant, exciting, but also challenging task that the field is currently facing.

While no consensus currently exists regarding the most relevant animal model of OA, it is clear that each type of model offers advantages and disadvantages that need to be taken into consideration. Spontaneous models best mimic the slow progression of the human disease, but they are time-consuming to use and the progression of the disease is usually quite variable between individuals (as in humans). Genetically modified mice constitute the best tools for mechanistic studies aiming at understanding the functional role of specific molecules in cartilage homeostasis and OA pathology, but their physiologic relevance to the human disease is questionable and their use as drug-screening tools remains to be validated. Surgically and enzymatically induced models develop rapid and reproducible damage, but might be more

Table 1 Animal models used to study OA and key recent findings

Type of model	Species	Salient findings/new functions
Surgically induced		
Anterior cruciate ligament transection	Mouse [22•,23], rat [35•], rabbit [87••], dog [60], cat [19•]	First model transposition to the mouse, early bone changes, chondro-protective effects of FGF-2
		Role of advanced glycation end-products in OA predisposition, differences between cats and dogs
Menisectomy	Mouse [22•], ewe [28], guinea pig [43••], rat [88•]	First model transposition to the mouse, detrimental effect of excess of NO
		Etiological role of anterior cruciate ligament, chondro-protective effect of FGF-18
Ovariectomy	Rat [24], ewe [26•]	Transposition of this postmenopausal model to the rat, detrimental role of NO
Articular groove	Dog [29•]	New model in which OA is induced by cartilage defects
Partial and full cartilaginous thickness defect	Rabbit [89], rat [90••]	Age-dependent repair capacity of FGF-2, repair ability of muscle-derived stem cells expressing BMP-4
Transarticular impact to induce trauma	Dog [30]	New model in which OA is induced through a bone defect
Enzymatically/chemically induced		
Collagenase induced	Mouse [44]	Mediation of osteophyte formation by synovial macrophages
TGF-β	Mouse [45]	Role of macrophages in TGF-β–induced osteophyte formation
Iodoacetate injection	Mouse [46,49]	Validation of a pain model
Spontaneous		
STR/ort	Mouse [54]	Role of ROS
Natural aging	Rat [57•], mouse [83•]	ATP depletion, mitochondrial impairment, age-dependent decrease in TGF-β signaling
	Guinea pig [43••,58], ewe [26•]	Role of ROS, etiological role of anterior cruciate ligament
Genetically modified		
ADAMTS-5 knockout	Mouse [65••,66••]	ADAMTS-5 is the major aggrecanase in OA
Del1 transgenic	Mouse [69]	Implication of cathepsin K in cartilage degradation
Cathepsin K transgenic	Mouse [72•]	Role of cathepsin K in synovitis
BMP receptor type 1a	Mouse [74]	Requirement of BMP signaling for adult cartilage homeostasis, first joint-specific genetically modified mouse
Mig	Mouse [75••]	Role of Mig-6 and control of cell proliferation in cartilage homeostasis
Biglycan/fibromodulin knockout	Mouse [76,77•,79]	Small proteoglycans are required for joint homeostasis
IL-6 knockout	Mouse [82••]	Protective role of IL-6
Cho/+	Mouse [96•]	How a type XI collagen defect leads to MMP-13 induction
IL-1β, IL-1β-converting enzyme, inducible NO synthase	Mouse [23]	Positive effect of cytokine
Drugs/supplements		
Alendronate	Rat ACLT [40]	Chondro-protection and prevention of osteophyte formation
PD-0200347	Dog [99•]	A ligand of the Ca^{2+} channel which slows down OA
S-34219	Guinea pig [100•]	Demonstration that a dual inhibition of MMPs and aggrecanases is needed for OA treatment
Pioglatazone	Guinea pig [101•]	A peroxisome proliferator-activated receptor γ agonist slows down OA
Vitamin C	Guinea pig [107]	Differential effects of vitamin C between spontaneous and menisectomized-driven OA

BMP, bone morphogenetic protein; FGF, fibroblast growth factor; IL, Interleukin; MMP, matrix metalloproteinase; NO, nitric oxide; OA, osteoarthritis; ROS, reactive oxygen species; TGF, transforming growth factor.

relevant to the traumatic forms of OA than to the classical degenerative form of OA. Chemically induced models develop even more rapid damage, but their physiological relevance is debatable. Most types of models have been characterized in several species (mouse, rat, guinea pig, rabbit, cat, dog, sheep), increasing the number of models available. Between species, for the same model, the rate of disease progression usually increases as the size and lifespan of the animal decreases – a characteristic that could reduce the response to treatment of the smaller species.

In the absence of a structure-modifying drug clearly effective in humans that could be used to evaluate the relevance of the existing animal models, compounds with demonstrated structure-modifying effects in animals (reviewed here and in [1]) could be used. Comparing the effects of these compounds on different animal models would offer us valuable comparative information on the response of these models to therapy and, ultimately, on their suitability as preclinical models as well as on their overall relevance to human OA. Such studies would allow for clarification of whether models developing fast damage or, on the contrary, slow damage are the most appropriate to be used as preclinical models for structure-modifying therapies. Both types of models were recently recommended as ideal preclinical models [35•,24]. Early onset and severe models are most economical and would probably lead to a lower number of false positives, but could also result in a high number of false-negative compounds as they constitute a high-hurdle test for detecting structure-modifying effects. Slow-onset models are more expensive and time-consuming, but would probably lead to a lower number of false-negative compounds as they mimic more closely the human disease. Such comparative studies would also clarify whether any single model can be used to evaluate the efficacy of a compound on traumatic and degenerative OA or if two models are necessary. For example, in the Hartley guinea pig, the same dose of vitamin C increased the severity of spontaneous knee OA [107], but decreased the severity of surgically induced knee OA [108] – a first indication that important differences in therapeutic response exist between spontaneous and surgical models, and that different models might be needed for degenerative OA and OA induced by injuries.

Conclusion

Studies on at least 25 different models of OA have been reported in the last 2 years (see Table 1). They provide new models for pain research as well as new basic information about the cell and molecular basis for OA, including the role of specific enzymes, growth factors and matrix proteins. They also provide new insights into the tissue origin of the disease. Collectively, they bring us closer but not yet reaching the 'Holy Grail': a gold standard model of OA.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 571–572).

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